



**Sudan University of Science and Technology**  
**College of Graduate Studies**



**Detection of Glucose-6-Phosphate Dehydrogenase Deficiency in  
Neonates with Jaundice at Khalifa Hospital, Ajman, United  
Arab Emirates**

الكشف عن نقص انزيم فوسفات الجلوكوز السداسي منتزع الهيدروجين عند حديثي  
الولادة المصابين باليرقان في مستشفى الشيخ خليفة في عجمان - دولة الإمارات  
العربية المتحدة

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of M.Sc. in Hematology and Immunohematology

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## الآية

وَقُلْ اِعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَىٰ عَالَمِ  
الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ ﴿١٠٥﴾

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بسم الله الرحمن الرحيم

﴿إِنَّ الَّذِي فَرَضَ عَلَيْكَ الْقُرْآنَ لَرَادُّكَ إِلَىٰ مَعَادٍ قُلْ رَبِّي أَعْلَمُ مَنْ جَاءَ  
بِالْهُدَىٰ وَمَنْ هُوَ فِي ضَلَالٍ مُّبِينٍ﴾

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## **Dedication**

The entire work is dedicated to my family, my friends, and my teachers at Sudan University for Science and Technology

## **Acknowledgement**

With a deep gratitude I acknowledge the support of my colleagues, without their efforts this work will never see the light. I also acknowledge the support of my supervisor Dr. Babiker Ahmed Mohammed Ahmed without his guidance, support and care this work will never reach completion. I also thank Mr. Muddather for his active participation, communication and training skills supported me in all steps preparing this thesis. And to my supervisors and managers at Sheik Khalifa Hospital in Ajman, Dr. Yasser Al Mudres.

## Abstract

Glucose 6-phosphate dehydrogenase (G6PD) is an enzyme which is catalyzed the first step in hexomonophosphate pathway of glucose metabolism. G6PD is the most common hemolytic X-linked genetic disease. This was cross sectional study conducted at Sheikh Khalifah Hospital, Ajman – United Arab Emirates. During the period January-2015 to December-2015 which is aimed to detection G6PD among neonates with jaundice and to find possible correlation between G6PD and Homological test (CBC, DAT, Retic)

And CRP as inflammatory parameter and total bilirubin as indicator of jaundice.

Sample collected in EDTA and heparinized tubes for investigations and the results analyzed using SPSS version (2015).

The present study shows that from 461 neonates participate in the study after approval from parents. (249 / 461), (54%) were male, while female were (212/461) (46 %)

G6PD deficiency found in 40 neonate (40/461) (8.6%) and male were more affected compared with female. (28/40)(70%), (12/40) (30%).

Other findings includes Hb in g/dl was 6-22 with average 14g/dl, regardless to the positively or negatively towards G6PD deficiency. Reticulocytes count average from lowest count (0.6 to 7.0)

Total WBC was estimated ration from low count (3.000 to 20.000) in cases deficient for G6PD, however, for the other cases is markedly variable from (3.000 to 111.000) which was not noted among deficient cases.

About the direct agglutination test (DAT) there were only two positive cases 5% the rest were negative 95% in G6PD deficient neonates. However the same result in the rest 421 cases.

CRP showed extremely variation in results. The lower estimates low 0.1 to 20 mg/L

Total bilirubin: results showed frequently comprises from (87 to 375mmol/L) among deficient cases

While among other hemolytic cases the results also look the same from less (40 to 400).

This study supports the importance of implementing G6PD screening for neonates as there are no laboratory parameters specific for indicating G6PD deficiency. However, the importance of G6PD screening lies in the prevention of future hemolysis and complications.

## المستخلص

ان انزيم فوسفات الجلوكوز ناقص الهيدروجين هو الانزيم المحفز للخطوة الأولى في مسار أحادي الفوسفات السداسي و الذي يختص بعملية استقلاب الجلوكوز ويؤدي نقص هذا الانزيم الى الإصابة بالمرض الوراثي الانحلالي الأكثر شيوعا المرتبط بطفرة جينية واقعة على الكروموسوم (X) و المعروف ب (التفول)

أجريت هذه الدراسة المقطعية في مستشفى الشيخ خليفة- عجمان - دولة الامارات العربية المتحدة خلال الفترة من يناير 2015 الى ديسمبر 2015 و التي تهدف الى الكشف عن نقص هذا الانزيم في المواليد الجدد المصابين باليرقان، كما هدفت الى إيجاد العلاقة بين مرض التفول و فحوصات الدم (CBC,Retic,DAT)،و كذلك العلاقة بين مرض التفول و بروتين سي التفاعلي (CRP) كمعامل للالتهاب و تحليل البيليروبين كمؤشر للصفار

جمعت العينات في انبوبي اختبار (EDTA , Heparin) و اجريت الفحوصات السابقة الذكر عليها. ونتائج تلك الفحوصات تم معالجتها بواسطة برنامج الحزمة الإحصائية للعلوم الاجتماعية (SPSS) اصدار (2015)

أجريت الدراسة على (461) طفل من حديثي الولادة بعد أخذ موافقة الوالدين و أظهرت النتائج أن من بين (54%) (249/461) كانوا ذكورا ،بينما (46%) (212/461) كانوا إناثا حدد نقص الانزيم (التفول) في 40 حالة من الأطفال حديثي الولادة بنسبة (8.6%) (40/461) كان أكثرهم من الذكور بنسبة (70%) (28/40) بينما نسبة إصابة الإناث (30%) (12/40) نسبة الهيموجلوبين بوحدة (g/dl) كان بين (6-22) بمعدل 14g/dl بغض النظر عن ايجابية أو سلبية النتيجة الخاصة بالتفول. معدل تعداد كريات الدم الحمراء الشبكية (Retic) بين (0.6-7) اجمالي تعداد كريات الدم البيضاء كان بين (20000-3000) في حالات نقص الانزيم (التفول) ولكن في الحالات الأخرى كان تعداد بين (11000-3000) و الذي لم يتم ملاحظتها في أطفال التفول و بخصوص فحص مضاد الجلوبيولين وجد حالتين ايجابيتين فقط (5%) و باقي الحالات كانت نتيجتها سلبية (95%) ما بين الأطفال المصابين بالتفول و كذلك نفس النتيجة و جدت في باقي ال (421) حالة

أظهرت نتيجة بروتين سي التفاعلي (CRP) اختلافات شاسعة ما بين 0.1-20. أظهرت نتيجة نسبة البيليروبين في كثير من الأحيان القيم ما بين (87 الى 375) ما بين حالات التفول و كذلك حالات فقر الدم الأخرى أظهرت نتائج مشابهة ما بين (40 الى 400).

أوصت هذه الدراسة بدعم أهمية ادخال فحص انزيم (G6PD) لدى حديثي الولادة حيث لا توجد دلالات مخبرية محددة تشير الى نقص انزيم (G6PD) كما أن أهمية هذا الفحص تكمن في الوقاية من المضاعفات التي قد تؤدي الى تحلل الدم في مراحل النمو المختلفة في المستقبل.

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## Abbreviations

AHG	Anti-human globulin
AIHA	Autoimmune hemolytic anemia
CBC	Complete Blood Count
CPDA-1	Citrate phosphate dextrose adenine
CRP	C- Reactive Protein
DAT	Direct Antiglobulin test
EDTA	Ethylenediaminetetraacetic acid
ETCO	End-Tidal carbon monoxide
FAD	Flavin adenine dinucleotide
G6PD	Glucose-6-Phosphate Dehydrogenase
Hb	Hemoglobin
HCT	Hematocrit
HDN	Hemolytic disease of the newborn
ICU	Intensive care unit
IV	Intravenous therapy
LDH	Lactate dehydrogenase
MSDS	Material Safety Data Sheet
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (Hydrogen)

OPIM	Other Potentially Infectious Material
PCV	Packed cell volumes
PPEs	Personal Protective Equipment's
QC	Quality Control
QNS	Quantity Not Sufficient
RBC	Red blood cell
Ret	Reticulocyte
ROS	Reactive Oxygen Species
SKH	Sheikh Khalifa Bin Zayed Hospital
SPSS	Statistical Package for the Social Science
TcB	Transcutaneous bilirubin
TSB	Total serum bilirubin
UAE	United Arab Emirates
UGT	Uridinediphosphoglucuronateglucuronosyltransferase
UV	Ultraviolet
WBC	(White Blood Cell) Count
WHO	World Health Organization

# Chapter one

## Introduction and literature reviews

### 1. Introduction

#### 1.1.1. Biochemistry of Glucose-6-Phosphate Dehydrogenase (G6PD)

As a historical background, G6PD was first isolated from *L. mesenteroides* fermenting bacteria by De Moss et al. in 1951 and later demonstrated its dual nucleotide specificity in 1953 and in 1955 showed that fermentation by *L. mesenteroides* proceeds via a different mechanism from the classical fermentation scheme. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that involved in regulation that catalyzing the first step of the pentose phosphate pathway: oxidation of glucose-6-phosphate using NADP<sup>+</sup> and/or NAD<sup>+</sup> based on the equation below:



NAD or NADP serves as coenzyme with the intrinsic reaction velocity of NAD being approximately 1.8 times greater than that of NADP. The *L. mesenteroides* G6PD gene encodes a polypeptide of 485 amino acids. G6PD is unique in that it contains no cysteine residues. The Molecular Weight is approximately 108.6 kDa which reacts in optimal pH of 7.8 and its Active Site Residues are Aspartic acid (D177) and Histidine (H240), (Worthington Enzyme Manual, 1993). G6PD Plays a Key Role in Protection Against Reactive Oxygen Species (ROS). These ROS generated in oxidative metabolism inflict damage on all classes of macromolecules and can ultimately lead to cell death. Indeed, ROS are implicated in a number of human diseases. Reduced glutathione, a tripeptide with a free sulfhydryl group, is required to combat oxidative stress and maintain the normal

reduced state in the cell. Oxidized glutathione is reduced by NADPH generated by glucose 6-phosphate dehydrogenase in the pentose phosphate pathway. Indeed, cells with reduced levels of glucose 6-phosphate dehydrogenase are especially sensitive to oxidative stress. This stress is most acute in red blood cells because, lacking mitochondria without any alternative averages of generating reducing power. (Berg *et al.*, 2002).

### **1.1.2. The Role of G6PD and G6PD Deficiency and Hemolysis**

At its introduction in 1926, an ant malarial drug, pamaquine, was associated with the appearance of severe and mysterious ailments. Most patients tolerated the drug well, but a few developed severe symptoms within a few days after therapy was started. The urine turned black, jaundice developed, and the hemoglobin content of the blood dropped sharply. In some cases, massive destruction of red blood cells caused death. This drug-induced hemolytic anemia was shown 30 years later to be caused by a deficiency of glucose 6-phosphate dehydrogenase, the enzyme catalyzing the first step in the oxidative branch of the pentose phosphate pathway. This defect, which is inherited on the X chromosome, is the most common enzymopathy, affecting hundreds of millions of people. The major role of NADPH in red cells is to reduce the disulfide form of glutathione to the sulfhydryl form. The enzyme that catalyzes the regeneration of reduced glutathione, the flavoprotein glutathione reductase, a dimer of 50-kd subunits, is homologous to ferredoxin-NADP<sup>+</sup>reductase, which we encountered in photosynthesis (Wahed and Dasgupta A, 2015). The reduced form of glutathione serves as a sulfhydryl buffer that maintains the cysteine residues of hemoglobin and other red-blood-cell proteins in the reduced state. Normally, the ratio of the reduced to oxidized forms of glutathione in red blood cells is 500. How is glutathione regenerated from glutathione reductase and NADPH by glutathione reductase? The electrons from NADPH are not directly transferred to the disulfide bond in oxidized glutathione. Rather, they are

transferred from NADPH to a tightly bound flavin adenine dinucleotide (FAD) on the reductase, then to a disulfide bridge between two cysteine residues in the enzyme subunit, and finally to oxidized glutathione. Reduced glutathione is essential for maintaining the normal structure of red blood cells and for keeping hemoglobin in the ferrous state (Wahed and Dasgupta, 2015). The reduced form also plays a role in detoxification by reacting with hydrogen peroxide and organic peroxides. Cells with a lowered level of reduced glutathione are more susceptible to hemolysis. It is therefore explained by due to the presence of pamaquine, a purine glycoside of fava beans, or other nonenzymatic oxidative agents' leads to the generation of peroxides, reactive oxygen species that can damage membranes as well as other biomolecules. Peroxides are normally eliminated by glutathione peroxidase with the use of glutathione as a reducing agent (Hurley, 2007). Moreover, in the absence of the enzyme, the hemoglobin sulfhydryl groups can no longer be maintained in the reduced form and hemoglobin molecules then cross-link with one another to form aggregates called Heinz bodies on cell membranes. A membrane damaged by the Heinz bodies and reactive oxygen species become deformed and the cell is likely to undergo lysis. In the absence of oxidative stress, however, the deficiency is quite benign. The occurrence of this dehydrogenase deficiency also clearly demonstrates that atypical reactions to drugs may have a genetic basis (Valencia *et al.*, 2016).

### **1.1.3. Glucose-6-Phosphate Dehydrogenase (G-6-PD) Deficiency**

G6PD deficiency is the most common enzymopathy affecting more than 400 million people worldwide. To date, there are roughly more than 400 variants have been described based on their biochemical properties and grouped into five classes according to the level of residual enzyme activity and clinical manifestations. Several variants considered different based on their biochemical properties, were shown to be identical at the molecular level.

Molecular analysis of the X-linked gene for G6PD identified 191 mutations or combination of mutations (Minucci *et al.*, 2012).

The function of Glucose-6-phosphate dehydrogenase is to catalyze the initial step in the hexose monophosphate (HMP or pentose phosphate) shunt, oxidizing glucose-6-phosphate to 6-phosphogluconolactone and reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. The HMP shunt is the only red cell source of NADPH, a cofactor important in glutathione metabolism (Glader, 2016).

G6PD deficiency was discovered as a result of a series of investigations performed to understand why some persons were uniquely sensitive to the development of hemolytic anemia when they ingested the 8-aminoquinoline antimalarial drug primaquine. Although many other red blood cell (RBC) enzyme deficiencies are now known, G6PD deficiency still reigns as the most common of all clinically significant enzyme defects, not only in hematology, but in human biology as a whole. A variety of drugs and infections cause hemolytic anemia in persons with the deficiency, and nonhematologic sequelae has been claimed as well (Beutler, 2008) and (Valencia *et al.*, 2016).

G6PD deficiency was discovered as an outgrowth of an investigation of hemolytic anemia occurring in some individuals treated for malaria with 6-methoxy-8-aminoquinoline drugs. Cordes reported the occurrence of acute hemolysis in such patients in 1926, but 3 decades passed before the mechanism of hemolysis could be understood. The discoveries that led to the recognition of G6PD deficiency were the result of several convergent events. First, the biochemical pathways through which red cells metabolize sugar were painstakingly unraveled by giants such as Warburg, Embden, and Meyerhof. Dr. Ernest Beutler has been recorded as the founder and the discoverer of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency as a genetic defect that leads to the lysis of red blood cells under conditions of



oxidative stress. This work hinged on his demonstration that red blood cell glutathione was unstable to oxidative stress. Later, he was to develop an assay for glutathione that was widely used in studies of red cell oxidative metabolism. Until now the most original information on G6PD deficiency support that Ernest Beutler was the main discovered of this genetic condition (Beutler,2014). It has been recognized for a long time that 8-aminoquinoline compounds may cause hemolytic anemia in certain individuals, but until recently the mechanism of such sensitivity has remained obscure. The use of modern hematologic techniques for the study of primaquine sensitivity has resulted in the discovery of a new intrinsic red cell defect. Cells with this defect are sensitive to hemolysis by a large number of aromatic amino compounds, including primaquine and other 8-aminoquinoline derivatives. G6PD is a critical enzyme in the production of glutathione, which defends red cell proteins (particularly hemoglobin) against oxidative damage. This X-linked disorder predominantly affects men. More than 300 G6PD variants exist worldwide, but only a minority cause hemolysis. Overall G6PD screen is important for better management of infants with disease (Beutler and Luzzatto,2014).

Most patients have no clinical or laboratory evidence of ongoing hemolysis until an event infection, drug reaction or ingestion of fava beans causes oxidative damage to hemoglobin. The oxidized and denatured hemoglobin cross-links and precipitates intracellularly, forming inclusions that are identified as Heinz bodies on the supravital stain of the peripheral smear. Heinz bodies are removed in the spleen, leaving erythrocytes with a missing section of cytoplasm; these “bite cells” can be seen on the routine blood smear. The altered erythrocytes undergo both intravascular and extravascular destruction. Older red blood cells are most susceptible, because they have an intrinsic G6PD deficiency coupled with the normal age-related decline in G6PD levels (Howes *et al.*, 2017). Hemolysis occurs two to four days

following exposure and varies from an asymptomatic decline in hemoglobin to a marked intravascular hemolysis. Even with ongoing exposure, the hemolysis usually is self-limited, as the older G6PD-deficient cells are destroyed. There is no specific therapy other than treatment of the underlying infection and avoidance of implicated medications. In cases of severe hemolysis, which can occur with the Mediterranean-variant enzyme, transfusion may be required (Beutler,2008 ; Al-Abdi, 2017).

Acute hemolysis is caused by infection, ingestion of fava beans, or exposure to an oxidative drug. Many medications that cause complications such as antimalarial drugs should be avoided in patients with G6PD deficiency. Hemolysis occurs after exposure to the stressor but does not continue despite continued infection or ingestion. This is thought to be a result of older erythrocytes having the greatest enzyme deficiency and undergoing hemolysis first. Once the population of deficient erythrocytes has been hemolyzed, younger erythrocytes and reticulocytes that typically have higher levels of enzyme activity are able to sustain the oxidative damage without hemolysis. Clinically, acute hemolysis can cause back or abdominal pain and jaundice secondary to a rise in unconjugated bilirubin. Jaundice, in the setting of normal liver function, typically does not occur until greater than 50 percent of the erythrocytes have been hemolyzed (Dhaliwal *et al.*,2004). G6PD activity levels may be measured as normal during an acute episode, because only non-hemolyzed, younger cells are assayed. If G6PD deficiency is suspected after a normal activity-level measurement, the assay should be repeated in two to three months, when cells of all ages are again present (Steensma *et al.*, 2001).

#### **1.1.4. Pathophysiology**

G6PD catalyzes nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form, NADPH, in the pentose phosphate pathway (WHO 1993). NADPH protects cells from oxidative damage. Because erythrocytes do not generate NADPH in any other way, they are more susceptible than other cells to destruction from oxidative stress. The level of G6PD activity in affected erythrocytes generally is lower than in other cells. Normal red blood cells that are not under oxidative stress generally exhibit G6PD activity at approximately 2% of total capacity. Even with enzyme activity that is substantially reduced, there may be few or no clinical symptoms. A total deficiency of G6PD is incompatible with life. The G6PD-deficient variants are grouped into different classes corresponding with disease severity (Mason, 1996). The gene mutations affecting encoding of G6PD are found on the distal long arm of the X chromosome. More than 400 mutations have been identified, the majority being missense mutations. Most of the variants occur sporadically, although the G6PD Mediterranean and the G6PD A-variants occur with increased frequency in certain populations (Al-Abdi, 2017).

## **1.2. Literature Review:**

### **1.2.1. Hemolytic Anemia Background**

The American Family Physicians Association defines Hemolytic anemia as a result of hemolysis which is the premature destruction removal of red blood cells from the circulation before their normal life span of 120 days. While hemolysis can be a lifelong asymptomatic condition, it most often presents as anemia when erythropoiesis cannot match the pace of red cell destruction i.e. if bone marrow activity cannot compensate for the erythrocyte loss (Dhaliwal *et al.*, 2004; Al-Abdi, 2017). The severity of the anemia depends on whether the onset of hemolysis is gradual or abrupt and on the extent of erythrocyte destruction. Mild hemolysis can be asymptomatic while the anemia in severe hemolysis can be life threatening and cause cardiovascular complications. Hemolysis also can manifest as jaundice, cholelithiasis, or isolated reticulocytosis (Schick, 2016).

### **1.2.2. Hemolysis**

There are two mechanisms of hemolysis these include intravascular hemolysis is the destruction of red blood cells in the circulation with the release of cell contents into the plasma. Intravascular hemolysis occurs in hemolytic anemia due many factors example Prosthetic cardiac valves, Thrombotic thrombocytopenic purpura, Disseminated intravascular coagulation , Transfusion of ABO incompatible blood , Paroxysmal nocturnal hemoglobinuria (PNH) and Glucose-6-phosphate dehydrogenase (G6PD) deficiency (Al-Abdi, 2017). The more common extravascular hemolysis is the removal and destruction of red blood cells with membrane alterations by the macrophages of the spleen and liver. A normal red blood cell can deform itself and pass through the 3-micron openings in the splenic cords. Red blood cells with structural alterations of the membrane surface

(including antibodies) are unable to traverse this network and are phagocytosed and destroyed by macrophages (Yang *et al.*, 2016).

Hemolysis may also be intramedullary, when fragile red blood cell (RBC) precursors are destroyed in the bone marrow prior to release into the circulation. Intramedullary hemolysis occurs in pernicious anemia and thalassemia major (Gong *et al.*, 2017).

Hemolysis is associated with a release of RBC lactate dehydrogenase (LDH). Hemoglobin released from damaged RBCs leads to an increase in indirect bilirubin and urobilinogen levels.

A patient with mild hemolysis may have normal hemoglobin levels if increased RBC production matches the rate of RBC destruction. However, patients with mild hemolysis may develop marked anemia if their bone marrow erythrocyte production is transiently shut off by viral (parvovirus B-19) or other infections.

### **1.2.3. Genetics and Hemolysis**

Chronic hemolysis can be a characteristic of disorders of hemoglobin synthesis, including sickle cell anemia and thalassemias. These include a heterogeneous group of inherited multifactorial anemias characterized by defects in the synthesis of the alpha or beta subunit of the hemoglobin tetramer ( $\alpha_2\beta_2$ ) (Yang *et al.*, 2016). The deficiency in one globin chain leads to an overall decrease in hemoglobin and the intracellular precipitation of the excess chain, which damages the membrane and leads to clinically evident hemolysis in the severe forms of alpha thalassemia (hemoglobin H disease) and beta thalassemia (intermedia and major). Beta thalassemia can be diagnosed by hemoglobin electrophoresis, which shows elevated levels of hemoglobins A2 and F, while diagnosis of alpha thalassemia requires genetic studies. Thalassemias are characterized by hypochromia and microcytosis; target cells frequently are seen on the peripheral smear (Maedel and Sommer, 1993) and (Howes *et al.*, 2017).

#### **1.2.4. Hyperbilirubinemia**

Bilirubin is defined as a tetrapyrrole and a breakdown product of heme catabolism. Most bilirubin (70%-90%) is derived from hemoglobin degradation and, to a lesser extent, from other hemo proteins; therefore its increased level reflects red blood cells destruction. In its unconjugated form, bilirubin is water-insoluble and binds avidly to tissues such as brain, sclera, and mucous membranes (Gong *et al.*, 2017). This is minimized by its binding to albumin in the plasma, which keeps it confined to the vascular space. The glomerular apparatus in the kidneys does not filter it. In the liver, the albumin-bilirubin complex dissociates, and it is taken up by the hepatocytes. It is conjugated via uridine diphosphoglucuronate glucuronosyltransferase (UGT) into its water-soluble form (Wehbi,2017) , (Yang *et al.*, 2016).

Bilirubin diglucuronide is the predominant conjugated form (80%-85%). Conjugated bilirubin is excreted into bile and delivered to the small intestine. Medical conditions and drugs that interfere with the excretion result in conjugated hyperbilirubinemia.

Intestinal bacteria convert bilirubin into several urobilinogens. A portion of the urobilinogens are then reabsorbed by the intestine and circulated back to the liver in a process called enterohepatic circulation. A small portion of urobilinogen is excreted from the body through the urine, while most is excreted in the stool. They give urine and stool their characteristic yellow and brown colors, respectively. In the event of absent urobilinogens in the stool and urine, the feces turn clay in color and the urine assumes a dark discoloration (SarkerSuprovath *et al.*, 2016).

Unconjugated hyperbilirubinemia can result from increased production, impaired conjugation, or impaired hepatic uptake of bilirubin, a yellow bile pigment produced from hemoglobin during erythrocyte destruction. It can

also occur naturally in newborns. Unless treated vigorously, most patients with Crigler-Najjar syndrome type 1, a form of unconjugated hyperbilirubinemia, die in early infancy. Overproduction of bilirubin (hemolysis) or defects in uptake and conjugation can result in unconjugated hyperbilirubinemia (Memon *et al.*, 2015).

Jaundice is the most common symptom of hyperbilirubinemia and is typically seen once total bilirubin levels approach 2-3 mg/dL. The earliest anatomic sites where jaundice can be seen are under the tongue and in the sclera (scleral icterus). Asymptomatic jaundice is common in ineffective erythropoiesis or hemolysis. In Gilbert syndrome, unconjugated bilirubin levels are mildly elevated at baseline but increase in the state of illness, physical or emotional stress, and in fasting (Dani *et al.*, 2015).

Dark urine is a primary presentation of conjugated hyperbilirubinemia, but not unconjugated hyperbilirubinemia, as it is water-insoluble and thus not excreted in the urine. Signs of ascites, splenomegaly, spider angiomas, and gynecomastia are typical of chronic liver disease. The presentation of neurological symptoms can indicate alcohol use. Tumors and an enlarged gallbladder may be evident as palpable abdominal masses. Several physical clues may suggest certain disorders, such as Kayser-Fleischer ring in Wilson disease or hyperpigmentation in hemochromatosis (Yang *et al.*, 2016).

### **1.2.5. Neonatal Jaundice**

Neonatal Jaundice has first been described in a Chinese textbook 1000 years ago, which is defined as a yellow coloration of the skin and sclera in newborns with jaundice is the result of accumulation of unconjugated bilirubin. In some infants, serum bilirubin levels may rise excessively, which can be cause for concern because unconjugated bilirubin is neurotoxic and can cause death in newborns and lifelong neurologic sequelae in infants who survive know as kernicterus jaundice. In 1875, Orth first described yellow

staining of the brain, in a pattern later referred to by Schmorl as kernicterus (Hansen,2017). Neonatal jaundice affects up to 84% of term newborns<sup>1</sup> and is the most common cause of hospital readmission in the neonatal period. Severe hyperbilirubinemia in which total serum bilirubin [TSB] level reaches more than 20 mg per dL or 342.1  $\mu\text{mol}$  per L. For these reasons, the presence of neonatal jaundice frequently results in diagnostic evaluation or even screening of jaundice is important especially with regard to G6PD (Sgro *et al.* , 2006).

The American Academy of Pediatrics recommends universal screening with TSB or transcutaneous bilirubin (TcB) levels, or targeted screening based on risk factors published in 2004. Universal TSB/TcB screening can accurately identify infants whose TSB level is likely to exceed the 95th percentile for age. Although screening can identify infants whose TSB level will likely exceed the 95th percentile, the U.S. Preventive Services Task Force and the American Academy of Family Physicians found insufficient evidence that screening for hyperbilirubinemia is associated with improved clinical outcomes (Al-Abdi, 2017). Screening will identify infants earlier who require phototherapy, but there is no evidence that phototherapy or exchange transfusion decreases the risk of bilirubin encephalopathy. Universal screening increases phototherapy rates, possibly inappropriately (Chung and Kulig, 2004).

#### **1.2.6. G6PD Deficiency Methods of Diagnosis**

The diagnosis of G6PD deficiency is made a quantitative analysis being performed using spectrophotometric or by a rapid fluorescent spot test detecting the generation of NADPH from NADP (Gregg and Prchal, 2000). The test is positive if the blood spot fails to fluoresce under ultraviolet light. Most newly available tests based on polymerase chain reaction detect specific mutations and are used for population screening, family studies, or prenatal diagnosis (Al-Abdi, 2017). In patients with acute hemolysis, testing



for G6PD deficiency may be falsely negative because older erythrocytes with a higher enzyme deficiency have been hemolyzed, because the young erythrocytes and reticulocytes have normal or near-normal enzyme activity, therefore it is important to avoid false negative test. Because of the difference in male and female disease inheritance, female heterozygotes may be hard to diagnose because of X-chromosome mosaicism leading to a partial deficiency that will not be detected reliably with screening tests (Jalloh A, Tantular IS, Pusarawati S, Kawilarang AP, Kerong H, Lin K, 2004). G6PD deficiency is one of a group of congenital hemolytic anemias, and its diagnosis should be considered in children with a family history of jaundice, anemia, splenomegaly, or cholelithiasis, especially in those of Mediterranean or African ancestry (Hermiston and Mentzer, 2002 ; Yang *et al.*, 2016).

Testing should be considered in children and adults (especially males of African, Mediterranean, or Asian descent) with an acute hemolytic reaction caused by infection, exposure to a known oxidative drug, or ingestion of fava beans. That why we aimed to conduct this study for the importance of screening infants to exclude G6PD deficiency. Although rare, G6PD deficiency should be considered as a cause of any chronic nonspherocytic hemolytic anemia across all population groups. Newborn screening for G6PD deficiency is not performed routinely, although it is done in countries with high disease prevalence. Therefore measuring prevalence is important to establish screening programs in the future (Gong *et al.*, 2017). The World Health Organization recommends screening all newborns in populations with a prevalence of 3 to 5 percent or more in males, this will further be considered in our discussion (WHO, 2010).

The prevalence of neonatal hyperbilirubinemia is twice that of the general population in males who carry the defective gene and in homozygous females. It rarely occurs in heterozygous females. This supports the

importance to investigate the magnitude of this problem in the daily practice (Kaplan *et al.*, 2001).

The mechanism by which G6PD deficiency causes neonatal hyperbilirubinemia is not completely understood. Although hemolysis may be observed in neonates who have G6PD deficiency and are jaundiced, other mechanisms appear to play a more important role in the development of hyperbilirubinemia. Hyperbilirubinemia is likely secondary to impairment of bilirubin conjugation and clearance by the liver leading to indirect hyperbilirubinemia (Al-Abdi, 2017). Infants with G6PD deficiency and a mutation of uridine diphosphoglucuronate glucuronosyltransferase-1 gene promoter (UDPGT-1) are particularly susceptible to hyperbilirubinemia secondary to decreased liver clearance of bilirubin. UDPGT-1 is the enzyme affected in Gilbert disease (Kaplan *et al.*, 2001). G6PD deficiency should be considered in neonates who develop hyperbilirubinemia within the first 24 hours of life, a history of jaundice in a sibling, bilirubin levels greater than the 95th percentile, and in males who are defined at risk. G6PD deficiency can lead to an increased risk and earlier onset of hyperbilirubinemia, which may require phototherapy or exchange transfusion. In certain populations, hyperbilirubinemia secondary to G6PD deficiency results in an increased rate of kernicterus and death, as the frequency measures within this population and whereas in other populations this has not been observed. This may reflect genetic mutations specific to different ethnic groups (Valaes, 1997;Al-Abdi, 2017).

### **1.3. Rationale:**

Glucose-6-phosphate dehydrogenase deficiency causes silent complications among neonates, this problem of neonatal jaundice and hyperbilirubinemia in Sheikh Khalifa in Ajman, UAE is overwhelming and caused great concern among professionals involved in neonatology and because of the frequency of cases seen in Sheik Khalifa Hospital. To that end, this had led us to focus on the importance of implementation of G6PDd screening testing or program to combat this serious problem. We however find screening of neonates with hyperbilirubinemia is very important of daily care in obstetric and gynecology units in Sheik Khalifa Hospital using simple laboratory test for G6PD.

## **1.4. Objectives:**

### **1.4.1. General Objective:**

To detect Glucose-6-Phosphate Dehydrogenase Deficiency in Neonates with Jaundice at Khalifa Hospital, Ajman, United Arab Emirates

### **1.4.2. Specific Objectives:**

- To determine the frequency of G6PD Deficiency among jaundiced neonates
- To assess the quality of G6PD rapid test with other laboratory test to triage cases G6PD deficiency.
- To correlate between G6PD Deficiency and Hematological Tests (CBC,DAT,Retic)
- To correlate between G6PD Deficiency and CRP as inflammatory test.
- To correlate between G6PD Deficiency and Total Bilirubin as indicator of jaundice.

## **Chapter Two**

### **Materials and Methods**

#### **2.1. Study design:**

This was prospective, analytical and across sectional study.

#### **2.2. Study area and duration:**

This study was done in Sheikh Khalifa Bin Zayed Hospital (SKH), Ajman, UAE during the period January 2015 to December 2015.

#### **2.3 Study population:**

The study involved neonates in the first week presented with jaundice.

##### **2.3.1 Inclusion criteria:**

All neonates born in SKH with jaundice

##### **2.3.2 Exclusion Criteria:**

Neonates without jaundice

#### **2.4 Sample size and Analysis:**

##### **2.4.1. Sample size and sampling technique:**

A total of 421 samples from neonates with jaundice or hyperbilirubinemia were collected and tested G6PDd, Complete Blood Count, Reticulocytes and Platelets Count and CRP

#### **2.5 Specimen collection:**

Based on consent and research ethical considerations, all specimens were collected based on questionnaire. The specimens were taken from neonates who develop jaundice between the period of January and December 2015. There were 461 specimens and a quantitative measurement of G6PD was performed. The samples were collected in EDTA and Heparinized containers, in ICU and nursery. Venepuncture is the preferred method of blood sampling for term neonates, and causes less pain than heel-pricks.

## **2.6 Sample preparation and Procedures:**

### **Procedures:**

#### **2.6.1 Complete Blood Count (CBC) and Reticulocyte Count:**

##### **Principle:**

The Cell Dyn uses fluorescence laser differentiation (MAPSS technology).

Specimen:  $\geq$  1ml EDTA Blood specimen (Lavender-top tube).

#### **2.6.2 Direct Antiglobulin Test**

To provide guidance for blood bank technologist to detect of in vivo red cell sensitization and determine which protein is coating the RBCs particularly IgG and C3 d using the manual technique. The direct Coombs' test measures the presence of antibodies on the surface of red blood cells. The DAT may be performed for investigation of: Diagnosis of hemolytic disease of the newborn (HDN) Diagnosis of autoimmune hemolytic anemia (AIHA)

Investigation of red blood cell sensitization caused by drugs. Investigation of transfusion reactions. A DAT is required if an autocontrol is not done in the antibody screen and: Antibody identification is required and autocontrol cannot be done (e.g., limited volume of serum/plasma) Antigen typing of the patient red cells is required. The antiglobulin reagent used for DAT shall contain antibodies to IgG and C3d component of complement. The only exception is cord blood testing that may be performed with a monospecific anti IgG reagent. In case DAT is performed on clotted blood, this identifies complement on the red cell surface; the result shall be verified using EDTA sample. All reagents shall be used and controlled and according to suppliers' recommendations and procedures. Check suitability of the specimen. Perform patient history check. The DAT is positive when agglutination is observed either after immediate centrifugation or after centrifugation following room temperature incubation. Immediate reactions are seen with IgG coated red cell Complement may be more easily

demonstrated after incubation. The DAT is negative when no agglutination is observed at either test phase, provided the check cells are reactive.

### **2.6.3 G6PD Screening Test**

The G-6-PDH Deficiency Screen by Spot Test is a qualitative test is intended for the qualitative, visual fluorescence screening of G-6-PDH in whole blood. Samples which have been determined deficient or intermediate should be assayed by a quantitative G-6-PDH method.

G6PD deficiency in red cells has been demonstrated to be the basis for certain drug induced hemolytic anaemias. The majority of subjects who have demonstrated G6PD

#### **Test Principle:**

The G-6-PDH Screening test is a qualitative test performed by incubating a small amount of blood with glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate (NADP). Drops of the mixture are removed at 5-minute intervals, spotted on filter paper and then viewed under long-wave ultraviolet light. Fluorescence is clearly evident in mixtures prepared from normal blood, whereas deficient samples yield little or no fluorescence.

#### **Specimen:**

**Patient Preparation:** N/A

#### **Type:**

EDTA-anticoagulated blood.

#### **Equipment and Materials:**

**Equipment:** Manually done

**Materials:** G-6-PDH SCREENING TEST (Biotech- Trinity) kit contains:

Buffer solution

G-6-6PD Substrate

G-6-PD Controls : deficient , intermediate , and normal

**Preparation:**

G-6-PD substrate solution is prepared by reconstituting G6PD substrate vial with 2.0 ml buffer solution

Allow to stand for 1-2 min and then mix by inversion.

**Storage Requirements:** Store G-6-PD substrate at 2 – 8 °C

Store G-6-PD substrate solution is stable for 1 week stored at 2 – 8 °C

**Procedure:**

Into tube add 0.2 ml G6PD substrate solution and 0.01 ml of normal control

Mix by swirling and transfer a drop of mixture to filter paper (zero time)

Place normal control tube in 37 °C water bath and record time

Do the same for intermediate, deficient and patient samples

Transfer additional drops of controls and test to filter paper 5 and 10 min after Zero time applications and allow to dry for 15-20 min

Visually inspect dried spots under UV light. Record florescent intensity of each sample.

**Interpretation of test:**

Compare the amount of fluorescence in the 10 min spots of the sample with that of normal, intermediate and deficient control

**NORMAL:** moderate or strong fluorescence is observed after 5 min and strong fluoresces after 10 min

**Intermediate:** Weak fluorescence is observed after 5 min and moderate fluoresces after 10 min

**Deficient:** No fluorescence is observed after both 5 and 10 min

**References:** G-6-PDH SCREENING TEST (Biotech- Trinity) kit insert.

**2.6.4 CRP and Total Bilirubin**

Test analyzed automated by using senchron machine.



**2.7 Ethical clearance;** This research samples were collected and analyzed based on approval and agreement of Sheikh Khalifa Hospital managers and other appropriate subject-specific guidelines and have discussed with them with the other researchers involved in the project. Informed the Committee of any changes to the protocol of this project until successfully finished.

### **2.8 Statistical analysis**

Data entering and checking and analyzed using SPSS version 2015.

Data represented by mean  $\pm$ 1 frequency, *P-value* signed at level  $\leq 0.05$

## Chapter Three

### Results

#### 3. Results:

Blood samples of 461 neonates born with jaundice (during the first week), these samples were analyzed. Out of 461 neonates, 249 were males and 212 females. Of these cases 40 were deficient, 28 male neonates and 12 female neonates. These samples were analyzed for G6PD deficiency, direct and indirect bilirubin, WBCs, Hb and Hematocrit, reticulocyte count, blood group and direct agglutination test. All these samples data was entered the SPSS and analyzed accordingly. In 40 (8.7%) patients, fluorescence was not at the fifth and tenth minute of incubation that indicated deficiency of G6PD. Where the amount of fluorescence is compared in the 5 min spots of the sample with that of normal, 5 to 10 intermediate (intermediate are also considered as deficient as well) which further classified to moderate or strong fluorescence is observed after 5 min and strong fluoresces after 10 min and intermediate, weak fluorescence is observed after 5 min and moderate fluoresces after 10 min. The remaining 421 samples (91.3%) neonates present with bright fluorescence at the fifth minute of the test. These samples were the negative cases or showed no deficiency in G6PD. Of these cases also there were 220 males and 201 males with ratio. These negative cases were important for discussion and sampling size (Table 1). According to the fluorescence of G6PD activity measurement 40 (8.7%) were G6PD (table 2).

Of these 40 cases i.e. G6PD deficient cases, were categorized generally. Provided the main focus of this study is highlight on the importance of G6PD screening for neonates at the first week age. Therefore from these 40 samples there were 28 males and 12 females, with ration of 2.3:1

respectively. Other findings include Hbratio in g/dl was in average of 6 to 22 with average 14 g/l, regardless to the positivity or negativity towards G6PD deficiency this parameter was not significant indicator for G6PD deficiency at all. The findings among neonates with normal G6PD, the findings were 6g/dl as lowest result and 23 g/dl for highest result. Again, in G6PD deficient neonates, the Hb result was from 15 g/dl to 24 g/dl which is considered as an interesting and fundament findings where no significant noted. This support the importance of G6PD screening for neonates as there are no laboratory parameters specific for indicating G6PD deficiency. However, the importance of G6PD screening lies in the prevention of future hemolysis and complications.

Reticulocytes count average from lowest count 0.6 to highest 7.0 which extremely variable among all G6PD deficient cases. The sample frequency was also observed among the rest of 421 cases.

Total bilirubin as the primary clinical presentation where quantitativelaboratory findings were important, however our result showed that is frequently comprises from less 87 to 375 highest peak, however this result assume findings among deficient cases to G6PD while among other hemolytic cases the result also look the same from less some 40 to 400.

The direct bilirubin was also estimated as lowest 3.00 to 26, where the same ratio was also expressed by findings from non G6PD deficient cases.

Interestingly, Hematocrit was almost comprises high or normal level with no sign of hemolysis in G6PD deficient cases with result of 50 at lower level and 60 highest level with average 55. In rest of cases the result was approximately from 30 to 60 with resulted average 45.

TWBCs was estimated ration from low count 3.000 to 20.000 in cases deficient for G6PD, however, for the other cases is markedly variable from 3.000 to 111.000 which was not noted among deficient cases.

About the direct agglutination test (DAT) there were only two positive cases 5% and the rest were negative 95% in G6PD deficient neonates. However, the same result in the rest of 421 cases.

C - reactive protein was also estimated, however in both groups of neonates with G6PD deficiency and those with normal findings CRP result showed extreme variation in results and no clear significant. The lower estimates as low 0.1 to 20 – a marked not significant result.

Last but not the least is the distribution of nationalities among G6PD Deficient cases although the number is only 40 cases with ratio of 8.4% from all samples, but the result shown the majority of positive cases were from United Arab Emirates (UAE), followed by Yemen and rest from other countries

**Table (3.1) Hematological Profile among study population**

(This table explains expression of HG vs HCT vs Reticulocyte count vs DAT comparing ratio as high, intermediate and negative results in G6PD deficient and normal cases)

<b>Test</b>	<b>G6PD Deficient cases</b>	<b>G6PD normal cases</b>
HG	95% of cases high	95% of cases high
HCT	95% of cases high	95% of cases high
Ret	88% of cases intermediate	90% of cases intermediate
DAT	99% of cases –ve	90% of cases -ve

**Table (3.2): Relation between gender and G6PD**

(This table highlights number and ratio of positive G6PD deficient cases)

<b>G6PD Result</b>	<b>Male</b>	<b>Female</b>	<b>Total</b>
Deficient	28	12	40
Ratio	70%	30%	100%

**Table (3.3): Frequency of G6PD among study volunteers**

(Significant percentage of positive cases)A total of 461 samples where a significant result of G6PD is observed.The intermediates appear here are also turned positive of repeat – so are true positive

	<b>Cases</b>	<b>Number</b>	<b>Percent</b>
Findings	Normal	421	91.1
	Deficient	40	8.7
	Total	461	99.8

**Table (3.4): Relation between categories of G6PD and gender**

(Initial G6PD findings before confirmation of intermediate cases)The intermediates appear here are also turned positive of repeat – so are true positive

	Male	Female	
1:Normal,	220	200	421
2:Deficient,	28	6	34
3:Intermediate	0	6	6
Total	248	212	461

**Table (3.5): Percentage of male and female**

(male and female neonates in this study)

Variable	Number	Percent
Male	249	54.1
Female	212	45.9
Total	461	100.0

## **Chapter Four**

### **Discussion, Conclusion and Recommendations**

#### **4.1. Discussion:**

Based on the result of this present study, the prevalence of G6PD deficiency among hyperbilirubinemic neonates was 8.4 % only as shown in table 1. Al-Omran et al, found that there is a correlation between hyperbilirubinemia and G6PD deficiency, and found that the most prevalent risk factor for hyperbilirubinemia was G6PD deficiency; this was in agreement with our findings as we selected neonates based on high bilirubin level (Al-Omran et al, 2017). Our result also is full agreement with (Ohkura et al, 1984) who reported an incidence of 8.6 % of G6PD deficient hyperbilirubinemic neonates in Mazandaran and Gilan provinces of Iran. Hasan et al studied 1,046 patients tested, and about 42% were G6PD deficient and presented with hyperbilirubinemia and even with sample size supported our findings 8.4% from our 421 screened cases (Hasan *et al.*, 2017). Strong evidence for supporting our result was the study done by Abo El Fotoh and Rizk where they concluded that 8.9% neonates out of 202 neonates had G6PD deficiency, all are males (100%) and in our study there were 70% males with is in full agreement. There was significant positive correlation between the time of appearance of jaundice in days and G6PD levels in G6PD deficient cases (Abo El Fotoh and Rizk, 2016). However in our study the same observation was noted. Also with regard to male and female ratio in the present study our result as documented table 2, where male to female ration and from these 40 samples there were 28 males and 12 females, with ration of 2.3:1 respectively. This indicated that the prevalence is higher in males than in females also further large required to justify this result but compared a recent study in Egypt carried out by Abosdera and Almasry 2014, their

results showed that all G6PD deficient newborns were males thus there is no G6PD deficient female neonates, they reported that G6PD deficient females were not at increased risk in the development of neonatal hyperbilirubinemia (Abosdera and Almasry, 2014). Similarly another recent study by (Howes *et al.*, 2017) and (Al-Abdi, 2017) carried out on newborns with indirect hyperbilirubinemia in Taiwan reported that the prevalence of G6PD deficiency was higher in males than in females. Thus the prevalence of G6PD deficiency in males was significantly higher than females in this study.

Based on our study there was no evidence of hyperbilirubinemia as an indicator to G6PD deficiency or severity table 3. Comparing further jaundice and other related hematological findings there was no significant relationship was noted between the severity of jaundice and the hematological indices as RBCs, reticulocyte count or hemoglobin concentration. Such findings were also reported by (Kaplan *et al.*, 2001) who suggested that jaundice is thought to be secondary to reduced hepatic conjugation and excretion of bilirubin, rather than increased bilirubin production resulting from hemolysis. Except a one case study done by Al-Abdi where he found that prolonged indirect hyperbilirubinemia in a preterm boy with G6PD deficiency which is insignificant as only one case was included in this study (Cherepnalkovski *et al.*, 2015). We have also noted and hypothesized in the present study, although bilirubin estimation was the main guiding laboratory findings on which we depended on selecting neonates for this study, it was evident that these neonates still having their mothers blood and yet the time for hemolysis. Or still the time is quite short for exposure to factors triggers G6PD deficiency hemolysis and complications.

We further agreed with study from Egypt by (Omran *et al.*, 2017) where authors demonstrated that G6PD deficiency by itself is a risk factor for the



development of neonatal hyperbilirubinemia even without exposure to chemicals that might cause hemolysis. These findings have implied that the possible cause of neonatal hyperbilirubinemia was not directly related to hemolysis, but was secondary to reduced hepatic conjugation and excretion of bilirubin. Other supporting studies conducted by (Howes *et al.*, 2017; Al-Abdi, 2017; Sirdah *et al.*, 2016; Yang *et al.*, 2016; Barreto *et al.*, 2016) concluded the same findings and support to our study in correlation between G6PD deficiency and hyperbilirubinemia.

For hemoglobin estimation as shown on table 3, the result also almost the same compared neonates with normal G6PD and G6PD deficiency. This also explains the same findings as for bilirubin. It therefore average for G6PD deficient cases is 14 to 24 g/dl average 18.5 g/dl compare to 6 g/dl to 25 g/dl average 15.5 g/dl for neonates with normal G6PD. This also was not significant indicator for G6PD deficiency in many occasions.

The same findings for Hematocrit where G6PD deficient neonates showed lower result 36 and 62 with average 44 compared to 20 to 68 with sane average 44, which is absolutely no significant for suggesting G6PD deficiency.

Reticulocyte count also was 0.11 to 18 average 9 in neonates with normal G6PD and 0.7 and 8.0 Average 5 which is not significant al, the fact that comparing the number of normal cases 421 to G6PD deficient cases 40 gives extremely variable results not sufficient comparison and significance for G6PD screening.

For the direct agglutination test (DAT), the tested samples were 329 from 461 neonates of which 31 were G6PD deficient neonates and 298 neonates with normal G6PD. Positive cases in normal with ratio 5.5% and the rest 94.5% were negative for DAT. While in G6PD deficient cases tested for DAT 6% were positive and 94% were negative, the same proportion was estimated, therefore no significance for G6PD screening.

## 4.2. Conclusion

1. Neonatal hyperbilirubinemia is one of the most common problems and may result in catastrophic consequences and required prompt screening and diagnosis.
2. Our studies has shown moderate prevalence 8.4% which comparable to other experiences and considered a signification justification for magnitude of G6PD deficiency crisis in the Gulf and other countries. Now we are confidently noted the importance of G6PD screening using this cost effective and sensitive technique at least to triage cases for further investigations.
3. We have come with evident identified a low cost, rapid, and effective screening tool that can be used as a model for general pediatricians in identifying another risk factor for neonatal hyperbilirubinemia, G6PD deficiency, specifically in high-risk infants often seen in outpatient follow up appointments and even in private settings.
4. The present study illustrates that it is important for the hospital to implement G6PD screening test for neonates at least as a trial and cohort testing to support G6PD screening, like the current screening program and have changed their management when knowing of the diagnosis of G6PD deficiency.
5. Some of the most convincing evidence in support of newborn screening comes from areas of high prevalence, such as Asian, African, Mediterranean, and Middle Eastern countries as documented in literature. Prevalence rate of 8.4% G6PD deficiency among male newborns with hyperbilirubinemia in Ajman UAE significantly higher

( $p < 0.01$ ) compared with rate among neonates in UAE population and in other countries.

### **4.3. Recommendations:**

- There is a need further studies for understanding the clinical problems, and understanding of G6PD deficiency and to development a comprehensive picture including pathophysiology, biochemical, and then molecular genetics, population genetics, and fine structure of the molecule that is at the root of the whole problem of this neonatal problem.
- It is recommended that G6PD spot test showed acceptable sensitivity and specificity for G6PD activity, and useful as screening, cost effective and simple laboratory method to be implemented.
- This study recommends availability of an economical laboratory and rapid test is important in each obstetrics and pediatrics hospitals and facilities, especially for the low-resource and under developed countries.
- Therefore, we recommend selective screening with this simple procedure to be performed in all hyperbilirubinemia neonates of unknown cause.
- The study recommends more research and conference to providing guidelines for G6PD screening in neonates.
- Further studies are required using large sample sizes for monitoring the magnitude of the problem and to enhance the sensitivity of the result.
- Essential communication between researchers dealing with G6PD linked to international hematology, gynecology and pediatrics professional organizations for working as team facing the problem of G6PD deficiency.